> this Office action. Claims 16-34 and 66-69 are withdrawn from further consideration by the Examiner, 37 C.F.R. § 1.142(b), as being drawn to a non-elected invention. (Office Action; page 3, lines 12-16).

With this Amendment, Applicant confirms the provisional election with traverse of Group I, claims 1-15 and 35-65.

Claims Rejected Under Section 103

The Office Action has rejected pending claims 1-15 and 35-65 under 35 U.S.C. §103 based upon either U.S. Patent No. 6,103,518 to Leighton or U.S. Pub. No. US2002/0132246 to Kallioniemi et al. in view of either Irving et al. (J of Clin. Path. (1996)49:258-259) or Goldsworthy et al. (Mol. Carcinog (1999)25(2):86-91). Leighton and Kallioniemi et al. each disclose a device and a method of creating a tissue microarray with paraffin as an embedding matrix. Neither Leighton nor Kallioniemi et al. discloses a tissue microarrayer specifically adapted to be used with a frozen embedding matrix, a frozen tissue microarray or a method of creating a frozen tissue microarray. Irving et al. and Goldsworthy et al. each merely disclose the fact that tissue may be frozen. Neither Irving et al nor Goldsworthy et al. discloses using the frozen tissue to construct a frozen tissue microarray.

In independent claims 1 and 12, the Applicant has claimed a method for preparing **a microarray of frozen tissue** comprising the steps of providing a microarray block comprising a plurality of donor tissue samples embedded in a block of **frozen embedding material**, each of said donor samples having a known location in said block; sectioning said block to generate a section comprising portions of said plurality of donor samples, each portion of each donor sample at a different sublocation in the section corresponding to coordinates of the donor sample in the microarray block from which each portion was obtained; and placing said section on a substrate such that said portions at different sublocations are stably associated with said substrate, thereby generating said microarray. The invention provides **microarrays comprising a plurality of frozen tissues** and/or cell samples and methods of preparing and using the same. By using **frozen samples**, the microarrays provide optimal samples from which to detect the expression of both nucleic acids and proteins in high throughput parallel analysis. More specifically, the Applicant's invention provides a method of generating a **frozen tissue**

microarray from the starting material of individual **frozen tissue samples**. Neither Leighton, Kallioniemi et al., Irving et al., nor Goldsworthy et al., alone or in combination, teach or make obvious the Applicant's claimed invention. As such, Applicant respectfully requests reconsideration and allowance of pending claims 1-15 and 35-65.

When reading either Leighton or Kallioniemi et al., in view of either Irving et al. or Goldsworthy et al., the resulting disclosure does not suggest that frozen tissue may be used to create a frozen tissue microarray. The resulting disclosure does not suggest that comparable tissue microarrays can be produced by the Leighton or the Kallioniemi et al. invention with the use of frozen tissue samples as was created with the use of paraffin embedded samples (i.e., number of samples comprising an array, number of arrays produced, types of tissues available, etc.). Neither Leighton, Kallioniemi et al., Irving et al. nor Goldsworthy et al., alone or in combination, suggest or disclose that the Leighton or Kallioniemi et al. invention could be adapted to utilize frozen tissue samples. In addition, the combined references do not disclose, teach or suggest a method of preparing a microarray of frozen tissues and or cell samples from individual frozen samples. The combined references do not disclose a method of creating a recipient block of a frozen embedding matrix; the combined references do not disclose the generation of a frozen microarray block by the addition of various frozen donor samples to the recipient block; and the combined references do not disclose a method of sectioning the frozen microarray blocks for the purpose of generating a number of substantially similar frozen tissue microarrays.

In addition, there is no motivation to combine the references. Irving et al. and Goldsworthy et al. never mention a microarray, frozen or otherwise. The disclosure that a tissue may be stored in a frozen state, as is disclosed by Irving et al. and Goldsworthy et al., would not provide one the motivation to construct a frozen tissue microarray as disclosed and claimed by the Applicant. As such, neither Leighton, Kallioniemi et al, Irving et al., nor Goldsworthy et al., alone or in combination, obviate the Applicant's claimed invention. As such, Applicant respectfully requests reconsideration and allowance of claims 1-15 and 35-65.

The Office Action rejected claims 1-15 and 35-65 under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent No. 6,103,518 to Leighton , in view of Irving et al. (J of Clin. Path. (1996) 49: 258-259) stating:

Leighton teaches a method for constructing tissue microarrays (also referred to as "tissue Chips") comprising,

> "taking samples from a series of donor tissues, one at a time, using a hollow, preferably needlelike, donor punch and placing each sample sequentially in a recipient of complementary shape in a recipient material by a recipient punch, thereby forming an array of tissues in the recipient block. Each punch comprises a punch tube and an associated stylet guided within the diameter approximating that of the donor punch inner diameter, and is dimensioned for sliding within the punch tube. The process of forming a hole in a recipient material such as paraffin, taking a sample of tissue from a donor specimen, and planting this sample in the hole in the recipient material, is repeated until a tissue array is formed comprising hundreds of tissue samples arranged in assigned locations in the recipient material. (col. 7).
>
> "Once the desired number of tissue samples have been transplanted from the donor block(s) to the recipient block, the "tissue chips" can be formed by slicing the tissue array block into hundreds of consecutive thin sections of, e.g., 5 micrometers in thickness, by traditional means (i.e., microtomes such as Model Cut .sub. 4055.TM. by Olympus Corp. of Tokyo, Japan, etc.; see, e.g., U.S. Pat. Nos. 664,118; 2,292,973; 2,680,992; 3,420,130; 3,440,913; 3,496,819; 3,799,029; and 3,975,977) to create multiple nearly identical sections, with each of the donor cores then being represented as minuscule dots on an ordinary glass microscope slide. Analyses that may be performed on the donor specimens include immunological analysis, nucleic acid hybridization, and clinicopathological characterization of the specimen." (col. 13).

Leighton also teaches:

> "The sample punched from the donor tissue sample is preferably cylindrical, about 1-8 mm in length, with a diameter of from about 0.4 to 4.0 mm, preferably about 0.3-2.0 mm. The recipient punch is slightly smaller than the donor punch and is used to create a hole in a recipient block, typically made of paraffin or other embedding medium." (col. 7).

Leighton also teaches that the methods can be automated and information for each donor sample in the recipient block is stored in a database (col. 7). Leighton also teaches that this array can be used for many types of samples, including diseased samples (col. 1-4). It is also noted, that with respect to claims 54-65 (claims drawn to contacting the microarray with a molecular probe),

> Leighton teaches that the array made in his methods can be used in nucleic acid hybridization, which would inherent use a molecular probe for detection (e.g., determining which sublocation react).
>
> Leighton teaches that the tissue samples are embedded in a block of paraffin or other embedding material. Leighton does not specifically teach the use of frozen embedding material. (Office Action; Page 4-Page 5).

The Office Action continues:

> However, Irving teaches that storing pathological tissue or cell specimens in OCT embedding material (i.e., a frozen embedding material) "permits retrospective analysis of RNA from small amounts of stored pathological samples" (see abstract). In other words, Irving teaches that embedding samples in OCT embedding material produces high quality RNA (i.e., RNA is not likely to get degraded in OCT, as it would in paraffin embedding material) (pg. 258).
>
> In view of the teachings of Irving, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Leighton so as to have embedded tissue and/or cell samples in OCT embedding material, in order to have achieved the benefit of providing a higher quality RNA, which would help obtain better results when analyzing the tissue and/or cell samples in subsequent molecular analysis (such as expression analysis). (Office Action; Page 5-6).

As stated above, neither Leighton nor Irving et al., alone or in combination, teach or make obvious the Applicant's claimed invention. As such, Applicant respectfully requests reconsideration and allowance of pending claims 1-15 and 35-65.

"Obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art." M.P.E.P. 2143.01. "The test for an implicit showing is what the combined teachings, knowledge of one of ordinary skill in the art, and the nature of the problem to be solved as a whole would have suggested to those of ordinary skill in the art." *In re Kotzab,* 217 F.3d 1365, 1370, 55 U.S.P.Q.2d 1313, 1317 (Fed. Cir. 2000). See also *In re Fine*, 837 F.2d 1071, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988); *In re Jones*, 958 F.2d 347, 21 U.S.P.Q.2d 1941 (Fed. Cir. 1992); M.P.E.P. 2143.01.

To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on applicant's disclosure. *In re Vaeck,* 947 F.2d 488, 20 USPQ2d 1438 (Fed.Cir. 1991). (MPEP, 706.02(j)).

Leighton discloses a simple, robust and precise instrument for constructing tissue arrays. (See Leighton, Abstract). Leighton discloses:

> Block 4 is formed of paraffin or a like material. Individual recipient holes can be punched onto the block either immediately prior to cutting and planting the donor sample, or an entire grid pattern of recipient holes may be cored into the recipient block prior to harvesting samples from the donor block. However, **due to the amorphous nature of the waxy donor block**, it is preferred to punch the recipient holes immediately prior to transplanting donor samples, in order to ensure the highest possible degree of alignment. (Leighton; Col. 9, Lines 34-42)(Emphasis added).

> The instrumentation art has proposed various optical surface detection systems such as laser diode triangulation devices (e.g. Dynavision Inc. model SPR-02, and several others), or structured light patterns visualized by an operator (e.g. Coherent-Ealing catalog #31-0458). Both are expensive as well as bulky. Both also suffer from imprecision, **since the surface in question in the present invention may be paraffin or similar waxy compounds** which are highly translucent. (Leighton; Col. 10, Line 66-Col. 11, Line 12)(Emphasis added).

As shown in the above-referenced passages, Leighton does not disclose, anticipate or teach a method of preparing a microarray of frozen tissue and/or cell samples. Further, the Office Action states that Leighton does not specifically teach the use of frozen embedding material.

The Office Action stated that Leighton in view of Irving et al. renders the Applicant's invention obvious. Irving et al. merely discloses that tissues and cells may be frozen to form individual frozen samples. Irving et al. states:

> OCT embedded cryostat sections of stored pathological specimens of non-Hodgkin's lymphoma were used to provide RNA. (Irving; Abstract, Lines 1-3).
>
> Here, we report the use of cryostat sections as an alternative source of high quality RNA for visualizing CD44 and its variants in non-Hodgkin's lymphoma by reverse transcriptase/polymerase chain reaction (RT-PCR). (Irving; Page 258).

Irving et al. merely discloses retrieving RNA from a frozen sample. There is no motivation or suggestion in the reference to combine Leighton with Irving et al. Despite Irving et al.'s disclosure that individual tissues may be frozen, Irving et al. does not suggest that frozen tissue may be used to create a frozen tissue microarray. As such, Leighton in view of Irving et al. does not render the Applicant's invention obvious. Applicant respectfully requests reconsideration and allowance of claims 1-15 and 35-65.

The Office Action rejected claims 1-15 and 35-65 under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent No. 6,103,518 to Leighton, in view of Goldsworthy et al. (Mol. Carcinog (1999) 25(2): 86-91), stating:

> The teachings of Leighton are presented above. Specifically, Leighton teaches the preparation of a tissue microarray, wherein the tissue samples are embedded in a block of paraffin or other embedding material. Leighton does not specifically teach the use of frozen embedding material.
>
> However, Goldsworthy teaches that "frozen tissues yielded more RT-PCR product than did paraffin-embedded tissues" when analyzing liver tissue expression (see abstract and pg. 87). Goldsworthy concludes that one of the reasons for this that, "the longer exposure of the fresh tissues to the fixative before embedding in paraffin may have resulted in RNA degradation from endogenous RNases, resulting in lower amounts of amplifiable RNA" (pg. 90, second column). Goldsworthy results, like others in the art, further support the idea that better results of amplification of RNA from tissues are obtained by using methods other than paraffin blocks (pg. 90, second column). (Office Action; Page 6).

The Office Action continued:

> In view of the teachings of Goldsworthy, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Leighton so as to have embedded tissue and/or cell samples in a frozen embedding material, in order to have achieved the benefit of providing a higher quality RNA, which would help obtain better results when analyzing the tissue and/or cell samples in subsequent molecular analysis (such as expression analysis). (Office Action; Page 6).

Similar to the discussion above, Goldsworthy et al. does not cure the deficiencies of the Leighton reference. Further, no motivation can be found in the cited references to combine Leighton with Goldsworthy et al. As such, Applicant respectfully requests reconsideration and allowance of pending claims 1-15 and 35-65.

Like Irving et al., Goldsworthy et al. merely discloses that tissue and/or cells may be frozen. Goldsworthy et al. discloses:

> The tissue morphology in the frozen and paraffin-embedded tissue sections scored on a scale of 1-3, with 1 indicating poor, 2 fair, and 3 good. Factors used in evaluating morphology included preservation of cytoplasmic and nuclear detail as well as stain clarity and uniformity. The slides were scored without knowledge of the tissue fixation protocol. (Goldsworthy et al., page 88).
>
> We showed that precipitive fixation of frozen tissue sections was the best protocol for preservation of mRNA in LCM specimens, especially when using ethidium bromide detection of the PCR product. Conversely, cross-linking fixatives such as NBF and paraformaldehyde produced less signal for both *GAPDH* and *Mβ-2* amplicons. Frozen tissues yielded more PCR product than did paraffin-embedded tissues. (Goldsworthy et al., page 90).

Goldsworthy et al. does not disclose, teach or suggest a method of preparing a microarray of frozen tissues and or cell samples. Goldsworthy et al. discloses a comparison of frozen tissue samples and paraffin embedded tissue samples. As stated above, Goldsworthy et al. concluded that frozen tissues yielded more PCR product than did paraffin embedded tissues. Such a conclusion does not provide the motivation to create a frozen tissue microarray. Goldsworthy et al. never mentions a use of a microarray or a method of preparing a microarray. As such, Leighton in view of Goldsworthy et al. does not render the Applicant's invention obvious. Applicant respectfully requests reconsideration and allowance of claims 1-15 and 35-65.

The Office Action rejected claims 1-15 and 35-65 under 35 U.S.C. 103(a) as being unpatentable over Kallioniemi et al. (Pub. No. US 2002/0132246), in view of Irving et al. (J of Clin. Path. (1996) 49: 258-259), stating:

Kallioniemi teaches a method for making a tissue microarray:

"In a specific example, core tissue biopsies having a diameter of 0.6 mm and a height of 3-4 mm, were taken from selected representative regions of individual "donor" paraffin-embedded tumor blocks and precisely arrayed into a new "recipient" paraffin block (20 mm.times.45 mm). H&E-stained sections were positioned above the donor blocks and used to guide sampling from morphologically representative sites in the tumors. Although the diameter of the biopsy punch can be varied, 0.6 mm cylinders have been found to be suitable because they are large enough to evaluate histological patterns in each element of the tumor array, yet are sufficiently small to cause only minimal damage to the original donor tissue blocks, and to isolate reasonably homogenous tissue blocks.

With the adhesive film in place, a 4-8 .mu.m section of the recipient block is cut transverse to the longitudinal axis of the tissue cylinders (FIG. 5) to produce a thin microarray section 76 (containing tissue specimen cylinder sections in the form of disks) that is transferred to a conventional specimen slide 78. The microarray section 76 is adhered to slide 78, for example by adhesive on the slide. The film 74 is then peeled away from the underlying microarray member 76 to expose it for processing. A darkened edge 80 of slide 78 is suitable for labeling or handling the slide." (pg. 5, Figs. 1-10 and 15-17).

Kallioniemi teaches that the samples can be that the methods can be automated and information for each donor sample in the recipient block is stored in a database (pg. 5, for example). Additionally, Kallioniemi teaches microarray can be used for many types of samples, including diseased samples (pgs. 1-4, Ex. 1-14). Kallioniemi also teaches methods including contacting the microarray with a molecular probe (pgs. 1-4, for example).

Kallioniemi does not specifically teach the use of frozen embedding material. (Office Action; Page 7).

The Office Action continued:

However, Irving teaches that storing pathological tissue or cell specimens in OCT embedding material (i.e., a frozen embedding material) "permits retrospective analysis of RNA from small amounts of stored pathological samples" (see abstract). In other words, Irving teaches that embedding samples in OCT embedding material produces high quality RNA (i.e., RNA is not likely to get degraded in OCT, as it would in paraffin embedding material) (pg. 258).

In view of the teachings of Irving, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kallioniemi so as to have embedded tissue and/or cell samples in OCT embedding material, in order to have achieved the benefit of providing a higher quality RNA, which would help obtain better results when analyzing the tissue and/or cell samples in subsequent molecular analysis (such as expression analysis). (Office Action; Page 7-8).

Similar to Leighton in view of Irving et al., Kallioniemi et al. in view of Irving et al. does not disclose, teach or suggest the Applicant's claimed invention. As such, Applicant respectfully requests reconsideration and allowance of pending claims 1-15 and 35-65.

Similar to Leighton, Kallioniemi et al. teaches a method for making a tissue microarray. Like Leighton, as stated in the Office Action, "Kallioniemi et al. does not specifically teach the use of frozen embedding material." (Office Action; page 7). Kallioniemi et al. discloses a method for rapid molecular profiling of tissue or other cellular specimens by placing a donor specimen in an assigned location in a recipient array, providing copies of the array, and performing a different biological analysis of each copy (See Kallioniemi et al., Abstract). Kallioniemi et al. discloses:

> In operation, the rectangular container 31 is placed on platform 32 (FIG.1) with edges of container 31 abutting edge guides 34 to hold container 31 in selected position. A donor block 30 is prepared by embedding a gross tissue specimen (such as a three dimensional tumor specimen 62) in **paraffin**. (Kallioniemi et al; page 4, paragraph 60)(Emphasis added).
>
> Up to 1000 such tissues cylinders, or more, can be placed in one 20X45mm recipient **paraffin block**. (Kallioniemi et al; page 5, paragraph 65)(Emphasis added).
>
> The array block may be **warmed at 37°C. for 15 minutes** before sectioning, to promote adherence of the tissue cores and allow smoothing of the block surface when pressing a smooth, clean surface (such as a microscope slide) against the block surface. (Kallioniemi et al; page 5, paragraph 66).

Kallioniemi et al., like Leighton, does not suggest replacing paraffin with a frozen embedding matrix. Likewise, Irving does not suggest creating a microarray from frozen tissue samples—Irving merely discloses that individual tissue samples may be frozen. Neither Kallioniemi et al. nor Irving et al., alone or in combination, anticipate, disclose, teach or suggest the Applicant's claimed invention. As such, Applicant respectfully requests reconsideration and allowance of pending claims 1-15 and 35-65.

The Office Action rejected claims 1-15 and 35-65 under 35 U.S.C. 103(a) as being unpatentable over Kallioniemi et al. (Pub. No. US 2002/0132246), in view of Goldsworthy et al. (Mol. Carcinog (1999) 25(2): 86-91), stating:

> The teachings of Kallioniemi are presented above. Specifically, Kallioniemi teaches the preparation of a tissue microarray, wherein the tissue samples are embedded in a block of paraffin. Kallioniemi does not specifically teach the use of frozen embedding material.
>
> However, Goldsworthy teaches that "frozen tissues yielded more RT-PCR product than did paraffin-embedded tissues" when analyzing liver tissue expression (see abstract and pg. 87). Goldsworthy concludes that one of the reasons for this that, "the longer exposure of the fresh tissues to the fixative before embedding in paraffin may have resulted in RNA degradation from endogenous RNases, resulting in lower amounts of amplifiable RNA" (pg. 90, second column). Goldsworthy results, like others in the art, further support the idea that better results of amplification of RNA from tissues are obtained by using methods other than paraffin blocks (pg. 90, second column). (Office Action; Page 8).

The Office Action continued:

> In view of the teachings of Goldsworthy, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kallioniemi so as to have embedded tissue and/or cell samples in a frozen embedding material, in order to have achieved the benefit of providing a higher quality RNA, which would help obtain better results when analyzing the tissue and/or cell samples in subsequent molecular analysis (such as expression analysis). (Office Action; Page 9).

Similar to the discussion above, Goldsworthy et al. does not cure the deficiencies of the Kallioniemi reference. Further, no motivation can be found in the cited references to combine Kallioniemi et al. with Goldsworthy et al. As such, Applicant respectfully requests reconsideration and allowance of pending claims 1-15 and 35-65.

Conclusion

In summary, neither Leighton, Irving et al., Kallioniemi et al, nor Goldsworthy et al., alone or in combination, anticipate, disclose, teach, or make obvious the Applicant's claimed invention. As such, Applicant respectfully requests reconsideration and allowance of pending claims 1-15 and 35-65.

Applicant submits that all claims are allowable as written and respectfully requests early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney of record.

Respectfully submitted,

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MARKED-UP VERSION OF AMENDMENTS:

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Please replace the paragraph at page 40, lines 4 through 12 with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

In one aspect, a frozen microarray is contacted with a molecular probe (e.g., an antibody, nucleic acid, and/or aptamer probe) reactive with a biomolecule and the reactivity of the molecular probe is measured to provide an indication of the presence, absence, or form of the biomolecule. Reactivity can be any of: binding, cleavage, processing, and/or labeling, and the like. Preferably, reactivity of the molecular probe with test samples in the microarray is compared with reactivity of the molecular probe with one or more control samples on the same or a different microarray comprising a known amount and/or form of the biomolecule. Molecular profiling can be performed using a variety of techniques, such as immunohistochemistry, in situ hybridization, and the like, in parallel or simultaneously[,].

Please replace the paragraph at page 47, lines 6 through 19 with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

A sub-category of tumor antigens includes the oncofetal tumor antigens. The oncofetal tumor antigens alphafetoprotein and carcinoembryonic antigen (CEA) are usually only highly expressed in developing embryos, but are frequently highly expressed by tumors of the liver and colon, respectively, in adults. Other oncofetal tumor antigens include, but are not limited to, placental alkaline phosphatase (Deonarain et al., 1997, *Protein Eng. 10*: 89-98; Travers & Bodmer, 1984, *Int. J. Cancer 33*: 633-641), sialyl-Lewis X (adenocarcinoma, Wittig et al., 1996, *Int. J. Cancer 67*: 80-85), CA-125 and CA-19 (gastrointestinal , hepatic, and gynecological tumors; Pitkanen et al., 1994, *Pediatr. Res. 35*: 205-208), TAG-72 (colorectal tumors; Gaudagni et al., 1996, *Anticancer Res. 16*: 2141-2148), epithelial glycoprotein 2 (pan-carcinoma expression; Roovers et al., 1998, *Br. J. Cancer. 78*: 1407-1416), pancreatic oncofetal antigen (Kithier et al., 1992, *Tumor Biol. 13*: 343-351), 5T4 (gastric carcinoma; Starzynska et al., 1998, *Eur. J. Gastroenterol. Hepatol. 10*: 479-484,; alphafetoprotein receptor (multiple tumor types, particularly mammary tumors; Moro et al., 1993, *Tumour Biol. 14*: 11-130), and M2A (germ cell neoplasia; Marks et al., 1999, *Brit. J. Cancer 80*: 569-578). [o]

Please delete the paragraph at page 54, line 5. The deleted paragraph is shown below:

[What is claimed is.]